

The University of Chicago

HYPERTHYROIDISM AND BRAIN OXIDATIONS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1935

By
ROBERT A. COHEN

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Reprinted from
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HYPERTHYROIDISM AND BRAIN OXIDATIONS¹

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ONE FIGURE

Since 1896, when Magnus-Levy suggested that the thyroid hormone might act either directly on tissues or indirectly by way of the nervous system, numerous attempts have been made to locate its site and manner of action. The opinion now, perhaps, most widely held favors a direct action upon individual cells, but even in recent years action via the nervous system has received support. Krogh ('16) reported, for example, that complete denervation of the extremities eliminated much of the metabolic rise produced in frogs by desiccated thyroid, presumably due to loss of sympathetic activity; and Abderhalden and Wertheimer ('26) interpret the inhibitory effect of ergotamine upon the action of thyroxine as further evidence that the latter acts via the sympathetic. Most striking are the claims of Mansfeld and Horvath ('35) that the respiration of slices of kidneys from thyroxinized dogs is not increased above normal when the kidney has been denervated before hyperthyroidism was induced, and that the respiration of the frog gastrocnemius is increased a third when only the cut end of its nerve is dipped for some hours in Ringer containing thyroxin. These findings were interpreted to mean that the hormone is carried to parenchymal cells only along efferent nerve fibers.

Whatever additional role the nervous system plays in raising basal metabolism—by increased motor discharges to smooth

¹ Preliminary reports of this material appeared in *Proc. Soc. Exp. Biol. and Med.*, vol. 32, p. 1446, 1935, and *Cold Spring Harbor Symposia*, vol. 4, p. 194, 1936.

or skeletal muscle or by aiding local hormone action—it remains true that the respiration of individual cells is enhanced by thyroid, for an increased oxygen consumption by hyperthyroid tissues persists *in vitro*. (Gerard and McIntyre, '34; Scott, '35. The general literature is adequately treated by Gerard and McIntyre and by McEachern, '35.) Few attempts have been made to analyze the changes in cell catalytic systems induced by thyroid and responsible for this increased respiration. Tissues from thyroidectomized animals show, besides a lowered oxygen consumption, a diminished indophenol-oxidase activity (Dye, '33) and a decrease of succinic dehydrogenase (Dye and Maughan, '29); other recent work on glycolytic and oxidizing systems will be considered later.

The present experiments were undertaken in the hope of demonstrating which step (or steps) in the oxidation sequence is hastened by the thyroid hormone and what cell enzymes are increased in activity as a result of its action. The brain is especially appropriate to such a study since any increase in its activity must secondarily affect the entire organism and enhance the local action of the hormone on other tissues. Further, there is doubt as to the degree of thyroid influence on the brain. Brains of normal and hyperthyroid rats have, therefore, been compared in their ability to oxidize various substrates and the relative effects of specific inhibitors determined.

METHOD

Male white rats weighing from 160 to 190 gm. were fed a stock diet with or without 0.6 gm. of desiccated thyroid added daily for 16 to 19 days. The controls gained an average of 21 gm.; the hyperthyroid animals lost from 4 to 10 gm. in this period. When ready, an animal was guillotined; the whole brain rapidly removed, minced and suspended in phosphate Ringer at pH 7.38; and oxygen consumption measured in modified Warburg manometers at 37°C. (Gerard, '31). Substrates, dyes, inhibitors and combinations of them were added to the *brei* or tipped from a side arm of the manometer cup.

Because of great variations in the early readings (probably determined by the amount of substrate initially present) the brei was allowed to respire for 2 hours (Quastel and Wheatley, '32), at which time remarkably constant readings were obtained. Substrates were then tipped in and the oxygen consumption for the next 90 minutes determined. With few exceptions, each figure given is based on six to ten experi-

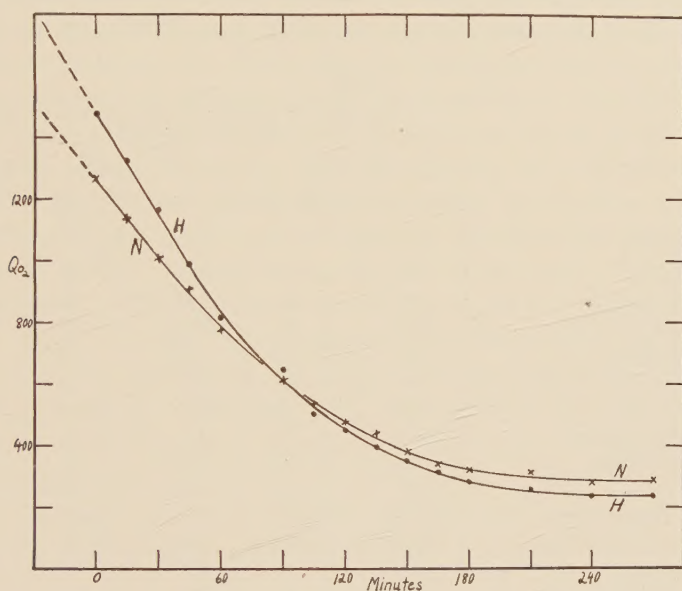


Figure 1

ments. Q_{O_2} values are per gram of fresh tissue; N refers to normal brain from control rats, H to that from hyperthyroid ones.

RESULTS

Residual respiration is initially 20% more in H than in N. It falls progressively but faster in H so that both are equal at 2 hours and H is 10% less in 4.5 hours. Extrapolating back to the time of removal, hyperthyroid brain in situ respire 30% more than the normal one. A typical experiment is shown in figure 1, which indicates the dependability of readings. Hyperthyroid brain, like other hyperthyroid tissues,

has a distinctly increased respiration. It follows that, in vitro, the limited substrate available will be sooner exhausted, and this accounts for the more rapid fall of Q_{O_2} for H than for N. Actually the total O_2 consumed by both is approximately the same, indicating a similar initial supply of substrate.

Oxidation of substrates. Hyperthyroid brain shows a selective increase in its power to oxidize various substrates. The Q_{O_2} of H is increased approximately four times as much as

TABLE 1

SUBSTRATE	RESPIRATORY RATE DURING 90 MINUTES AFTER TIPPING SUBSTRATE				
	Normal		Hyperthyroid		Per cent increase H/N
	Substrate	Per cent increase	Substrate	Per cent increase	
None—control	520		475		
M/10 glucose	620	+ 18	855	+ 81	4.4
M/10 Na lactate	625	+ 19	885	+ 86	4.4
M/100 Na succinate	1010	+ 92	2300	+ 385	4.2
0.1% glycogen	535	+ 3	530	+ 12	4.1
M/100 Na glycerophosphate	640	+ 22	825	+ 74	3.3
M/10 fructose	905	+ 72	1375	+ 190	2.6
M/100 Na pyruvate	875	+ 68	815	+ 72	1.1
M/100 galactose	685	+ 32	630	+ 33	1.1
M/200 } para-	1700	+ 235	1610	+ 240	1.0
M/100 } phenylene-	2900	+ 450	2640	+ 460	1.0
M/50 } diamine	3600	+ 600	4165	+ 780	1.3
M/10 glycine	580	+ 11	520	+ 10	0.9
M/100 methyl glyoxal	620	+ 19	530	+ 16	0.9

that of N on adding those substrates which are probably involved in carbohydrate oxidation and glycolysis (glycogen, glucose, fructose, glycerophosphate, lactate, succinate). This is true even though the absolute increases for these substances vary from 3 to over 90%. With p-phenylenediamine the increase for H is one-third greater than for N; while other substrates (pyruvate, galactose, glycine, methyl glyoxal) affect both alike (table 1). Clearly certain specific enzyme systems and not others are enhanced by the thyroid hormone.

Respiration accelerators. Methylene blue and cresyl blue have been shown to accelerate the oxygen consumption of

many tissues. By virtue of their easy reduction and re-oxidation (by atmospheric oxygen), they may provide a 'short cut' for the oxidation of certain substrates (notably lactate) ordinarily oxidized by respiratory intermediates, which latter in turn are reoxidized by air at a much slower rate than are the dyes. Whether or not a dye can act as a catalyst depends on whether the normal oxygen consumption by the cell is slower or quicker than the oxidation activated by the dye (Barron and Hoffman, '30. See also Gerard, '32, for a more complete discussion). Dyes might, therefore, have had quantitatively different effects on the respiration of H and N if a differential increase of certain systems occurred in H.

It was found, however, that methylene blue (0.01%) and cresyl blue (0.02%) effect H and N alike, leading to an early increase (30 and 25%, respectively, in 15 minutes) and a later decrease of Q_{O_2} (equal to control at 30 minutes, 10% less in 90 minutes). Stronger concentrations (0.04%) had an earlier toxic effect; weaker concentration (0.001%) gave no significant change although decoloration indicated that the dye was reduced (compare Dickens, '36). Cresyl blue (0.02%) cut the increase after tipping glucose or succinate to 80 to 70% of that occurring when the substrates were tipped alone, but again percentage values for H and N were the same. Any catalytic differential was apparently confused by the toxic action (see also Cohen and Gerard, '37, on methyl red). Alwall's ('36) positive results with the dinitrophenol group will be considered later.

Respiration inhibitors. The catalytic chain in cells for oxidation of substrate is composed generally of three types of links. Substrate is activated by its specific dehydrogenase (often with accessory co- and intermediate enzymes or carriers) at one end and yields hydrogen (or electrons) to a hydrogen acceptor. At the other end, oxygen, acting through the cytochromeoxidase,² accepts electrons from cytochrome or, acting directly, from flavoprotein or other intermediate donators. The dehydrogenase system may couple directly

² This name is more significant biologically than the more common indophenol-oxidase.

with the oxidase system through cytochrome, for example, or still other intermediate links (fumaric acid, acetoacetic acid, ascorbic acid, pyocyanine, etc.) may be intercalated. Cyanide poisons the cytochromeoxidase system and cresyl blue (etc.) can substitute for this step. Arsenite and narcotics block dehydrogenases but do not affect the oxidase end; since paraphenylenediamine oxidation requires only oxidase and cytochrome, it is not affected by these. Malonate blocks the succinic dehydrogenase specifically, and iodoacetate the oxidation of sugars by interference with aldolase and the triosephosphate dismuting enzymes.

The respiratory rate of any cell is limited, among other things, by the active catalysts present (see Gerard, '36, for a fuller discussion). The inhibition produced by a poison specific to one link in the chain would be greater or less as the catalytic activity at this step was relatively low or high in relation to other steps. Thus, if normally the oxidase and dehydrogenase reactions are equal, but after thyroid administration the dehydrogenase is increased beyond the oxidase, then in hyperthyroid brain as compared to normal narcotics should be relatively less inhibitory than is cyanide. This will be more fully discussed later, the point here is that a comparison of the decreases produced by various inhibitors in the oxidation of particular substrates by normal and by hyperthyroid brains can give information as to the relative enzyme activity in each.

An extensive series of such experiments is summarized in table 2 a and b. In 2 a inhibitor is added to brain plus substrate, in 2 b, substrate to brain plus inhibitor; and in each case the percentage change in respiration produced by the addition is indicated. Most significant, is the last column of each panel giving the ratio of percentage change in hyperthyroid brain to that in normal. Thus it can be seen that M/100 NaCN reduced the Q_{O_2} of N to 20% and that of H to 18% of its original value, giving $H/N = 0.9$. Similarly the increased respiration in the presence of glucose was reduced by 23 and 28%, $H/N = 1.2$; of succinate, to 12 and 11%; of p-phenylenediamine, to 22% for each. M/100 NaCN caused

TABLE 2

ADDED SUBSTRATE		M/1000 NaCN							M/1000 NaCN and 0.02% cresyl blue						
		Normal			Hyperthyroid				Normal			Hyperthyroid			
		Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N
a															
None		520	105	20	475	90	18	0.9	520	360	69	475	305	63	0.9
M/10 glucose		620	140	23	860	240	28	1.2	620	175	28	860	240	28	1.0
M/10 lactate		625			885										
M/100 succinate		1010	120	12	2300	250	11	0.9	1010	835	83	2300	1720	75	0.9
M/100 p-phenylene diamine		2880	635	22	2640	580	22	1.0	2880	780	27	2640	740	28	1.0
b		Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N
None															
M/10 glucose		105	190	185	85	240	275	1.5	105	235	225	85	220	255	1.1
M/10 lactate									105	255	225	85	240	275	1.2
M/100 succinate		105	195	190	85	255	295	1.6	105	1290	1240	85	1650	1900	1.5
M/100 p-phenylene diamine		105	510	490	85	490	560	1.2	105	525	505	85	610	700	1.4
a		M/100 Na iodoacetate							M/100 Na malonate						
		Normal			Hyperthyroid				Normal			Hyperthyroid			
		Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N
None		520	67	13	475	63	13	1.0	520	500	96	475	455	96	1.0
M/10 glucose		620	210	34	860	290	34	1.0							
M/10 lactate		625	75	12	885	195	22	1.0							
M/100 succinate		1010	210	21	2300	505	22	1.0	1010	310	31	2300	575	25	0.8
M/100 p-phenylene diamine		2880	2590	90	2640	2530	96	1.1	2880	3050	106	2640	2580	98	0.9
b		Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N
None															
M/10 glucose		67	245	365	63	270	430	1.2							
M/10 lactate		67	105	155	63	190	300	1.9							
M/100 succinate		67	285	425	63	475	755	1.8	500	670	134	455	630	139	0.9
M/100 p-phenylene diamine		67	1500	2230	63	1990	3150	1.4	500	1800	360	455	1920	424	1.2

TABLE 2—Continued

ADDED SUBSTRATE	M/1000 Na arsenite							0.1% barbital						
	Normal			Hyperthyroid				Normal			Hyperthyroid			
	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N
a														
None	520	140	27	475	140	29	1.1	520	490	94	470	445	93	1.0
M/10 glucose								620	640	103	860	875	102	1.0
M/10 lactate								625	580	93	885	790	89	0.9
M/100 succinate	1010	525	52	2300	1270	55	1.1	1010	805	80	2300	1840	80	1.0
M/100 p-phenylene diamine	2880	2890	100	2640	2690	102	1.0	2880	2850	99	2640	2730	103	1.0
b	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N
None														
M/10 glucose								490	595	122	450	825	183	1.5
M/10 lactate								490	800	164	450	810	180	1.1
M/100 succinate	140	760	540	135	990	730	1.3	490	610	125	450	1560	350	2.8
M/100 p-phenylene diamine	140	2700	1920	135	2950	2160	1.1	490	1700	345	450	1970	440	1.3
a	0.1% urethane							2.5% urethane						
	Normal			Hyperthyroid				Normal			Hyperthyroid			
	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N
None	520	505	97	470	475	101	1.0	520	110	21	470	120	26	1.2
M/10 glucose	620	620	100	860	860	100	1.0	620	75	12	860	155	18	1.5
M/10 lactate	625	950	152	885	1345	152	1.0	625	90	14	885	185	21	1.5
M/100 succinate	1010	1015	100	2300	2300	100	1.0	1010	1010	100	2300	2320	101	1.0
M/100 p-phenylene diamine	2880	2870	100	2640	2630	100	1.0	2880	2050	71	2640	2060	78	1.1
b	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N
None														
M/10 glucose	505	610	121	480	865	180	1.5	110	125	115	125	150	120	1.0
M/10 lactate	505	655	130	480	1145	240	1.8	110	145	131	125	185	148	1.1
M/100 succinate	505	980	195	480	2330	485	2.5	110	1865	1710	125	2360	1910	1.1
M/100 p-phenylene diamine	505	1770	350	480	2090	435	1.4	110	1625	1490	125	1540	1250	0.9

TABLE 2—*Continued*

ADDED SUBSTRATE	5.0% urethane						
	Normal			Hyperthyroid			
	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Substrate alone	Substrate + inhibitor	Per cent not inhibited	Not inhibited H Not inhibited N
a							
None	520	62	12	470	72	15	1.2
M/10 glucose	620	49	8	860	86	10	1.2
M/10 lactate	625	62	10	885	88	10	1.0
M/100 succinate	1010	1000	100	2300	2350	102	1.0
M/100 p-phenylene diamine	2880	950	33	2640	1025	39	1.2
b	Inhibitor alone	Inhibitor + substrate	Per cent increase	Inhibitor alone	Inhibitor + substrate	Per cent increase	Increase H Increase N
None							
M/10 glucose	62	7	11	72	8	11	1.0
M/10 lactate	62	105	167	72	130	187	1.1
M/100 succinate	62	1625	2620	72	2690	2740	1.4
M/100 p-phenylene diamine	62	625	1010	72	720	1000	1.0

almost complete inhibition; M/1000 NaCN caused an inhibition of only 10 to 15% in both N and H. Conversely, addition of substrate always increased oxygen consumption markedly even though an inhibitor was present. Thus glucose added to cyanide-inhibited brei increased the Q_{O_2} of N to 183%, of H to 275, giving H/N of 1.5.

The addition of 0.02% cresyl blue to cyanide-inhibited brei restored the Q_{O_2} to 69% in N and 63% in H; with glucose present to 28 and 28%, respectively; with succinate to 83 and 75%; and with p-phenylenediamine to 27 and 28%. The results with brei alone indicate that cresyl blue can restore the Q_{O_2} to two-thirds its normal value, the remaining inhibition possibly being due to a toxic effect of the cyanide on other enzyme systems than the oxidase, or to a toxic action of the dye. It is striking that cyanide inhibits the Q_{O_2} of brain alone,

or with added glucose or p-phenylenediamine, to about one-fifth, with added succinate to one-ninth; but dye addition restores little of the loss when glucose or p-phenylenediamine is the substrate, four-fifths when succinate is, and two-thirds for brei alone. Clearly these various substrates are not equally dependent on the cytochrome—cytochromeoxidase system for their oxidation or else the cresyl blue exerts some specific side effects. It is noteworthy, that a variety of oxidation inhibitors and accelerators have quantitatively the same action (in per cent) on oxidation of glucose and of p-phenylenediamine by brain.

Na iodoacetate (M/100) cut the respiration of both N and H alone to 13%; with glucose present, both fell to 34%; with lactate, to 12% in N and 22% in H; with succinate, to 21 and 22%; with p-phenylenediamine, to 90 and 96%, respectively. The action of this inhibitor is by no means clear. The results with p-phenylenediamine show a negligible effect on the oxidase system. It does block hexose degradation at the triose-phosphate stage, and also removes glutathione, which functions as a co-enzyme to methyl-glyoxalase and in the lactate-pyruvate system. Brei alone, or after lactate addition, is most inhibited—suggesting that lactate is the substrate for the residual respiration (Cohen and Gerard, '37). The smaller inhibition of glucose indicates that the poison has a greater effect beyond the lactate stage of glycolysis than before it; and the iodoacetate concentration used here is rather high so that other systems might also be poisoned. At least for nerve (Chang and Gerard, '33) and, with lower iodoacetate concentrations, for brain (Quastel and Wheatley, '32), lactate oxidation is relatively spared by this poison. The marked inhibition of succinate oxidation is of interest in relation to its possible intermediacy in carbohydrate oxidations (Krebs and Johnson, '37). Inhibition is alike in H and N except with lactate and the difference here may not be valid since only four experiments were run with each.

Sodium malonate (M/100) had little effect on the respiration of brei alone or with added p-phenylenediamine but abolished

two-thirds of the succinate oxidation. This inhibitor competes with succinic acid for the succinic dehydrogenase (Quastel and Wheatley, '31), and the results show that this enzyme is present in roughly equal proportion in both N and H.

Barbital and urethane act alike, presumably to inhibit dehydrogenases. Two and five-tenths per cent urethane, which gave most significant results, cut the respiration of brei alone to 21% in N and 26% in H; with succinate, to 100 and 101%; with p-phenylenediamine to 71 and 78%. Urethane, supposed not to act on the oxidase system, yet decreases the oxidation of p-phenylenediamine by 25%; and certainly it is acting on other systems, for the Q_{O_2} with glucose and with lactate is cut to 15%. Yet with succinate the Q_{O_2} is unchanged, and succinate requires both a dehydrase and the cytochrome-oxidase system (see also Jowett and Quastel, '37). This recalls the observation of Cohen and Gerard ('37) that succinoxidase activity is well preserved in a brain extract which has lost the power to oxidize glucose and lactate. Further, sodium arsenite, supposed also to be quite specific for dehydrogenases, cuts the respiration of brei alone to 27% in N and 29% in H; with succinate added, to 52 and 55%, while with p-phenylenediamine no inhibition results. Clearly narcotics and arsenite do not act identically nor does either simply inhibit dehydrogenases.

DISCUSSION

Weight for weight and (excluding changes in water content) probably cell for cell, thyroxinized brain burns substrates with oxygen at an augmented rate—30% or more above the normal. The same is true for body cells in general. Since oxidation velocities in cells are ordinarily independent of the concentrations of reactants or products (Gerard, '36), this must depend on an increase in cell enzyme activity—presumably in the actual number of enzyme molecules. (The possibility of changes in auxilliary accelerating or inhibiting molecules has been elsewhere considered, Gerard, '36). The increased catalytic activity, furthermore, is not in like proportion for all oxidations but is specific for certain ones. It follows that

individual cell catalysts have been increased under thyroxin action, others not at all or in smaller degree. So far as our limited exploration permits a generalization, those substances which occur in the sequence of carbohydrate oxidation or glycolysis are the ones more rapidly oxidized by hyperthyroid than by normal brain. Whatever the absolute rate in any instance, the speed of oxidation by H is about four times that by N. The absence of pyruvate (and methyl glyoxal) from this group is very interesting.

It is significant that the brain normally and even under a variety of extreme experimental conditions (see Gerard, '37, for literature) has an R.Q. of 1.0 and utilizes only carbohydrate and related substrates. Since, moreover, hyperthyroidism lowers the R.Q. for the entire body, it is far from certain that the hormone increases carbohydrate oxidation in all or most cells as it does in those of the brain. Possibly in each case oxidation of the favored substrate of the cell is hastened by increase of the specific dehydrogenases already most active. This can be decided by extending such experiments as these to other tissues and particularly by including R.Q. determinations. Should it turn out that thyroxin increases the concentration of different enzymes in different cells rather than exerting a like action on all, some important conclusions as to its mode of action and, indeed, on the synthesis of enzymes in general would follow.

Even on the present evidence it seems clear that the hormone, directly or after any sort of modification into another molecule, is not a necessary and slowest link in the chain of oxidation catalysts, as has been suggested (Kendall, '29). Were this so, cretin tissues should fail to carry on essential oxidations and hyperthyroid ones should execute all with greater dispatch. Further, as will be discussed, several different enzymes are increased by thyroxin.

A second theory (Weil and Landsberg, '29) makes the oxidation increase secondary to the accumulation of products of anaerobic reactions which are directly accelerated by thyroxin. An increased anaerobic glycolysis accompanying hyperthyroidism (Haffner, '27) has been reported for kidney but

not liver (Dresel, '28; Anselmino, Eichler and Schlossmann, '29; Ebina, '32; and McEachern, '35), for liver (Meyer, McTiernan and Aub, '33) and for neither (Reiss, Hochwald and Druckrey, '33). Since only kidney has shown increased glycolysis with any certainty while liver and muscle, etc., as well as kidney, increase their respiration under thyroid action, the glycolysis change can hardly be essential to that of respiration. Enhanced protein breakdown as the basis for the greater oxygen consumption is supported by Mansfeld and Horvath ('35) who find that ammonia formation is augmented by thyroid parallel with the respiration increase. They further explain the temporal lag in thyroid action as due to the time required for thyroxin to penetrate into the cell and stimulate hydrolyses, and they claim to make its action immediate by accelerating lytic processes by a brief period of anoxia or by treatment with carbylamine. Such theories imply more rapid oxidations consequent to increased substrate rather than increased enzyme concentration; and since the latter does result from thyroxin they can at best be but partially correct. Further, it is difficult to see how particular substrates (or products) are maintained in high concentration, especially in tissue brei *in vitro*, unless as a result of heightened activity of the catalytic systems which lead to their formation.

The conclusion seems probable, therefore, that the thyroid hormone acts by facilitating the formation of certain specific enzymes normally present in cells, as is in fact demonstrated, and it remains to consider which ones. An increase of one-third in the rate of oxidation of p-phenylenediamine is direct evidence of an increase in the cytochromeoxidase-cytochrome system. A more complete analysis follows.

The rate of substrate oxidation is dependent (with adequate O_2 and substrate concentrations) on the rates of concatenated reactions in the chain of intermediates. These may be conveniently grouped into an oxidase sequence up to, say, cytochrome, and a dehydrogenase sequence. For the former, the oxidase concentration is most important, for the latter that of dehydrogenases. Whichever step is slowest will limit the

entire oxidation rate. [The valuable analysis of Burton ('37) showing the limitations of the 'master reaction' principle, does not invalidate this statement. His strictures apply to reversible or irreversible reactions only when the reaction velocity of a given step depends on the concentration of reactants which can accumulate from a preceding step. This seems often not to be the case in biological oxidations—see, as instances, the oxidation of lactate by *Sarcina lutea* (Gerard, '31) or of succinate by brain (Cohen and Gerard, '37). If, for example, cytochrome is kept almost completely oxidized by the oxidase system, its reduction must determine rates; and interference with dehydrogenases will slow oxidations since no further piling up of oxidized cytochrome, to overcome the slowing, is possible.]

If one step is crucial to the oxidation rate, then hyperthyroid cells, with a higher rate, must have a more active catalytic system for this step, whether or not that for the other is also more active. The use of inhibitors of the oxidase or dehydrogenase, respectively, permits an analysis. An example may aid the argument.

Suppose in the normal cell the oxidase system permits a rate of 100, the dehydrogenase one of 50, which is, then, the respiratory rate. In the hyperthyroid cell, respiration is increased to 75 and the dehydrogenase likewise. The oxidase may remain at 100 or, if also increased by half, become 150. Suppose a given cyanide concentration blocks 50 units of oxidase in the normal. If it inhibits a fixed absolute number of oxidase units it will also block 50 in the hyperthyroid cell, but if it inhibits a fixed fraction of the total units it will block 75. The latter condition is rather more probable, a priori, but both are possible. The rate of oxygen consumption of the normal cell would not be affected by this amount of cyanide, since 50 units of oxidase remain to keep up with 50 units of dehydrogenase. The same would be true for the hyperthyroid cell if oxidase increased in proportion to dehydrogenase and if cyanide inhibited a fixed number (50) or a fixed fraction (75); for at least 75 of the 150 units of oxidase would remain active to match the

75 of dehydrogenase. But if oxidase was not increased, this same cyanide concentration would leave only 50 oxidase units active and so inhibit respiration by one-third (from 75 to 50).

In general, if oxidase does not increase in H, then a given CN concentration will inhibit H more than N and H/N will be less than 1 (i.e., $\frac{H^{inhib}}{H} / \frac{N^{inhib}}{N}$). If oxidase increases in proportion to dehydrase and CN inhibits a fixed percentage then, of course, H/N is always 1.0; but if CN inhibits a fixed number of units then H/N is greater than 1.0. The ratio of the respiratory fraction of inhibited over uninhibited H to that of N under the action of a given inhibitor can thus be interpreted in terms of the activity of two catalytic systems.

TABLE 3

INHIBITORS ASSUMED TO BLOCK ENZYME MOLECULES	PRESUMED CHANGE FROM NORMAL TO HYPERTHYROID BRAIN IN CONTENT OF		EXPECTED RATIO OF Q_{O_2} OF HYPER- THYROID TO NORMAL BRAIN (H/N) UNDER INHIBITION BY	
	Oxidase system	Dehydrase system	Cyanide	Narcotic
As a fixed	+	+	1	1
fraction of	+	0	1	< 1
total present	0	+	< 1	1
As a fixed	+	+	> 1	> 1
absolute	+	0	> 1	< 1
number	0	+	< 1	> 1

The possible conditions are summarized in table 3. When both systems are increased proportionally the shift in H/N is in the same direction which ever step originally set the pace. When only one is increased, obviously it must be the one initially limiting respiratory rate. It is noteworthy that, if an inhibitor acts upon a fixed fraction of enzyme units, H/N never exceeds 1.0, while if it blocks a fixed number, H/N is never 1.0 (except, of course, with weak inhibition) and mostly exceeds it. Examination of the actual results (table 4) indicates a predominant increase in dehydrogenase systems, despite certain inconsistencies. For brain without substrate addition, H/N is greater than 1.0 with adequate urethane dosage, less than 1.0 with cyanide. With lactate present, H/N is greater

than 1.0 with urethane, still more so with iodoacetate. The ratio for glucose is increased by urethane and also by cyanide, as if important increases in both oxidase and dehydrogenase occurred, but, somewhat surprisingly, it is not changed by iodoacetate. The failure of H/N for succinate to increase under narcotics is also unexpected in view of the great increase in succindehydrogenase in H. It can be assumed that in this case inhibition is of a fixed fraction of molecules, which fits the results, but this is not very satisfying.

In general, it seems fair to conclude that these results—both the absolute changes in rate of substrate oxidation and the relative effects of inhibitors—strongly favor a considerable increase in particular dehydrogenases and a lesser increase

TABLE 4

BRAIN + ADDED SUBSTRATE	H/N FOR INHIBITION BY		
	M/1000 NaCN	2.5% urethane	M/100 Na Iodoacetate
None	0.9	1.2	1.0
M/10 glucose	1.2	1.5	1.0
M/10 Na lactate	...	1.5	1.8
M/100 Na succinate	0.9	1.0	1.0
M/100 p-phenylene diamine	1.0	1.1	1.1

in oxidase in the hyperthyroid as compared to the normal brain.

Alwall ('36) has independently reached an equivalent position from a very different approach, the synergistic action of thyroid and nitrophenols on cell respiration. The increased oxygen consumption induced in hyperthyroid tissues by nitrophenol is far greater than the increase in normal ones. The acceleration is believed to be due to increase in the activity of hydrogen carriers between activated substrate and oxidase. These normally set the rate of oxidations and, if more rapid, permit an increased respiration. The dehydrogenase, however, then becomes the slow step and no great respiration increase is possible. Thyroxin, by enhancing dehydrogenases, leaves a wider margin of acceleration for the nitrophenol.

SUMMARY

1. The Q_{O_2} of hyperthyroid brain is initially 30% higher than that of normal, becomes equal to it in 2 hours, and falls to 10% below in $4\frac{1}{2}$ hours.

2. The Q_{O_2} of hyperthyroid brain is increased approximately four times as much as that of normal on adding substrates which are involved in carbohydrate oxidation and glycolysis (glycogen, glucose, fructose, glycerophosphate, lactate, succinate), even though absolute values for individual substrates vary widely. With p-phenylenediamine the increase over normal is 33%. With other substrates (pyruvate, galactose, glycine, methyl glyoxal) increases in hyperthyroid and normal brain are approximately equal.

3. Analysis of the respiration changes, induced by methylene blue and cresyl blue and such inhibitors as cyanide, malonate, iodoacetate, arsenite, barbital and urethane, indicates that the absolute concentration of various enzyme systems is greater in hyperthyroid than in normal brain and that certain dehydrogenases are increased relatively more than oxidases.

LITERATURE CITED

- ABDERHALDEN, E., AND E. WERTHEIMER 1926 Ernährung und Inkretwirkungen. VI. Thyroxin-Wirkung bei Verfütterung verschieden zusammengesetzter Nahrung. Arch. Ges. Physiol., Bd. 213, S. 328.
- ALWALL, N. 1936 Über die Wirkung der Dinitrophenole auf die tierischen Oxydationsprozesse. Skand. Arch. Physiol., Bd. 72, suppl. 1.
- ANSELMINO, K. I., O. EICHLER AND G. SCHLOSSMANN 1929 Über den Einfluss des Thyroxins auf den Stoffwechsel überlebender Gewebe. Biochem. Zeit., Bd. 205, S. 481.
- BARRON, E. S. G., AND L. A. HOFFMANN 1930 The catalytic effect of dyes on the oxygen consumption of living cells. J. Gen. Physiol., vol. 13, p. 483.
- BURTON, A. C. 1936 The basis of the principle of the master reaction in biology. J. Cell. and Comp. Physiol., vol. 9, p. 1.
- CHANG, T. H., AND R. W. GERARD 1933 Studies on nerve metabolism. VII. The role of lactic acid. Am. J. Physiol., vol. 104, p. 291.
- COHEN, M. B., AND R. W. GERARD 1937 Oxidizing enzymes in brain extracts. Am. J. Physiol., vol. 119, p. 34.
- DICKENS, F. 1936 Metabolism of normal and tumour tissues. XVI. Action of some oxidation-reduction systems. Biochem. J., vol. 30, p. 1064.
- DRESEL, K. 1928 Über das Wesen der Oxydationssteigernden Wirkung des Thyroxins. Klin. Wchnschr., Bd. 7, S. 504.

- DYE, J. A. 1933 The action of thyroxin on tissue respiration. *Am. J. Physiol.*, vol. 105, p. 518.
- DYE, J. A., AND G. H. MAUGHAN 1929 Tissue respiration and endocrine function. III. Influence of thyroidectomy on tissue respiration. IV. Influence of thyroidectomy on succinoxidase activity of surviving tissue. *Proc. Soc. Exp. Biol. and Med.*, vol. 26, pp. 439, 441.
- EBINA, T. 1932 Über den Wirkungsmechanismus des Thyroxins beim Gewebestoffwechsel. *Tohoku J. Exp. Med.*, Bd. 19, S. 139.
- GERARD, R. W. 1931 A modified Warburg apparatus and some applications. *Am. J. Physiol.*, vol. 97, p. 522.
- 1931 Observations on the metabolism of *Sarcina lutea*. II. *Biol. Bull.*, vol. 60, p. 227.
- 1932 Nerve metabolism. *Physiol. Rev.*, vol. 12, p. 469.
- 1936 Metabolism and excitation. *Cold Spring Harbor Symposia*, vol. 4, p. 194.
- 1937 Metabolism of brain and nerve. *Ann. Rev. Biochem.*, vol. 6, p. 419.
- GERARD, R. W., AND M. MCINTYRE 1933 The effect of thyroid feeding on tissue respiration. *Am. J. Physiol.*, vol. 103, p. 225.
- HAFFNER, F. 1927 Pharmakologische Untersuchungen mit einem deutschen Thyroxin. *Klin. Wchnschr.*, Bd. 6, S. 1932.
- JOWETT, M., AND J. H. QUASTEL 1937 The effects of narcotics on tissue oxidations. *Biochem. J.*, vol. 31, p. 565.
- KENDALL, E. C. 1929 Thyroxine. The Chemical Catalog Co., New York.
- KREBS, H. A., AND W. A. JOHNSON 1937 Metabolism of Ketonic acids in animal tissues. *Biochem. J.*, vol. 31, p. 645.
- KROGH, M. 1916 The influence of thyroidin on normal metabolism. *Ugesk. Laeger*, vol. 77, p. 2337.
- MANSFELD, G., AND G. HORWATH 1935 Die Rolle des Nervensystems bei der Wirkung des Thyroxins auf die Zellatmung. *Pflüger's Arch.*, Bd. 235, S. 520.
- MCEACHERN, D. 1935 The metabolism of isolated surviving tissues from animals rendered hyperthyroid with thyroxin. *Bull. Johns Hopkins Hosp.*, vol. 56, p. 145.
- MEYER, O., C. MCTIERNAN AND J. AUB 1933 Relation of internal secretions to tumor metabolism. *Endocrinology*, vol. 17, p. 363.
- QUASTEL, J. H., AND A. H. M. WHEATLEY 1931 Biological oxidations in the succinic acid series. *Biochem. J.*, vol. 25, p. 117.
- 1932 Oxidations by the brain. *Biochem. J.*, vol. 26, p. 725.
- REISS, M., A. HOCHWALD AND H. DRUCKREY 1933 Thyreotroper Wirkstoff des Hypophysenvorderlappens. *Med. Klin.*, Bd. 29, S. 1112.
- SCOTT, A. H. 1935 Thyroxin and tissue metabolism. *Am. J. Physiol.*, vol. 111, p. 107.
- WEIL, R., AND M. LANDSBERG 1929 Über die Wirkungswiese des Schilddrüsenhormone. *Biochem. Zeit.*, Bd. 207, S. 186.

